CANINE HEMATOPOIESIS IN A MODEL OF COMBINED INJURY

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INTRODUCTION

The aftermath of a nuclear accident or disaster will present another set of problems to the attending physicians and other medical personnel. They will be presented with alternatives not previously encountered in conventional warfare or in our urban trauma centers. The source of these alternatives or options is the combined-injury patient, that is, the physically injured or traumatized person who has also been irradiated. Each injury can be a serious problem under disaster conditions, but when combined, the single sublethal injury can be quickly transformed into a lethal one. The significance of the combined injury (CI) following a nuclear detonation is evident from the medical records of the casualties treated in Japan. Over 40% of the casualties from Hiroshima and Nagasaki suffered multiple injuries. Experimental studies conducted on various animal models have revealed that the combined effects of injury from more than one energy form or trauma are synergistic in effect. Brooks et al.[1] showed that in a combined insult of thermal burns following total-body irradiation, a dose of 25 r significantly increased lethality following a burn of 20% body area. Alpen and Sheline,[2] in a similar study using a rat model, showed that total-body X irradiation to a sublethal dose of 100 R in combination with an LD50 level of burn injury increased the lethality to 65%. Schildt and Thoren[3] have summarized the characteristics of the combined injury syndrome. It must be mentioned that in their case, the CI is defined as "a complex injury caused by a simultaneous exposure to two (or more) forms of energy or traumata of various kinds." Here it is not required for irradiation to be one of the traumas. Their characteristics include the synergistic effect, a moderation of reaction capacity, impaired wound healing, increased tendency toward shock, and increased susceptibility to complicating infections.

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In the context of this presentation, exposure to a sublethal dose of ionizing radiation is considered as the initial cause of the CI, followed by a second stressor either immediately or within several days postexposure. A substantial sublethal exposure to gamma or mixed neutron-gamma radiation severely damages the hemopoietic system of the mammalian species. Predisposition to bacterial sepsis by opportunistic pathogens and impaired wound healing are two of the major consequences of radiation-induced hemopoietic and immune suppression. Functional cells are decreased within days to critically low levels, and precursor and stem cells that are responsible for the regeneration of mature cells may be depressed for weeks after sublethal doses of irradiation.

The development of a large animal model for CI within the context of a nuclear disaster required that we describe, experimentally, the essential features of the radiobiology of acute effects in the canine. The large-animal model is also appropriate for assessing the immunologic, pharmacologic, and surgical modes of intervention following CI. The canine model of CI at the AFRRI has stressed three developmental aspects: (a) establishing the radiobiology of the canine hemopoietic system, (b) choosing a relevant model for peritoneal sepsis, and (c) identifying several choices for physical trauma. This paper stresses the relevance of the first aspect, the radiation-induced suppression and recovery of the hemopoietic system.

MATERIALS AND METHODS

ANIMALS. Healthy, pure-bred, male and female beagles (9-12 kg) were used in these studies. The dogs were treated to eliminate parasitic infections and were immunized against distemper, hepatitis, and rabies. They were observed for 2 weeks before they entered the experimental protocol. They were housed in temperature controlled rooms, in individual stainless-steel cages, and fed kibbled laboratory dog food, which was supplemented once a week with high-protein, canned-meat ration. Water was provided ad libitum.

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HEMATOLOGICAL VALUES AND HEMOPOIETIC CULTURE TECHNIQUES.

Peripheral blood was withdrawn from the cephalic vein and bone marrow (BM) was aspirated from the ribs and iliac crest of anesthesized dogs (Bio-Tal, Parke Davis, A.J. Buck & Son, Baltimore, MD) into heparinized syringes. Peripheral blood leukocytes and platelets were counted using a hemocytometer. Plasma from a 1-ml aliquot of blood was harvested for the assay of colony-stimulating activity (CSA). A 5-ml aliquot of blood was used for separation of mononuclear cells (PBMC) using Lymphocyte Separation Medium (LSM, Bionetics, Kensington, MD) and centrifuged at 1500 rpm (400 x g) for 35 min. The mononuclear cell (MNC) layer was harvested and counted for viable nucleated cells in a hemocytometer. BM-derived mononuclear cells were harvested in a similar manner. These cell populations were then assayed for specific hemopoietic progenitor cells.

Granulocyte-macrophage (GM-CFC) and macrophage (M-CFC) colony-forming cells were assayed using the double-layer agar technique as previously described.[4] Briefly, CSA was provided by using pooled plasma from dogs previously injected with Escherichia coli (O55:B5) lipopolysaccharide (List Biologicals, Campbell, CA), which was added to the bottom agar layer (7% vol/vol) of triplicate culture dishes. BM-derived MNC and PBMNC were plated in the upper agar layer in concentrations depending on previous treatment. Colonies (> 50 cells) counted after 10 days and 27 days of culture were considered to be derived from GM-CFC and M-CFC, respectively.

Erythroid progenitor cells (CFU-e) were assayed, using the plasma clot technique. For each cell sample, 2 ml of the plasma clot suspension was prepared to contain 0.6 ml heat-inactivated fetal bovine serum, 0.2 ml of 25% beef embryo extract, 0.2 ml of 10% bovine serum albumin, 0.2 ml (0.04 mg) L-asparagine, 0.2 ml of 10^{-3} M 2-mercaptoethanol, 0.2 ml (1 unit) sheep erythro-poietin (Ep) (Step III, Connaught Medical Research Labs., Swiftwater, PA), 0.2 ml cells (concentrations yielding 1×10^5 to 5×10^6 cells per clot), and 0.2 ml of 37^oC bovine citrated plasma. All ingredients were either reconstituted or diluted with supplemented alpha medium (SAM),[35] and control plasma clots contained SAM in place of Ep. Immediately, 0.5 ml of this mixture was

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pipetted into each of three 17-mm flat-bottomed wells in Linbro tissue culture plates, allowed to clot, and incubated for 72 hours at 37°C in a humidified atmosphere containing 5% CO_2 in air. Plasma clots were then harvested, fixed with 5% gluteraldehyde, stained with benzidine and giemsa, and scored at 25 X using a conventional light microscope.

PARAMETERS OF COBALT-60 AND MIXED NEUTRON-GAMMA IRRADIATION.

The canines were secured in Plexiglas holders for both types of exposure. Cobalt-60 irradiation was bilateral at a dose rate of 0.1 Gy per minute to various total-body, midline tissue-absorbed doses (MTD). Mixed neutron-gamma irradiation was achieved in a gadolinium-lined exposure room from the AFRRI TRIGA Mark-F pool-type thermal research reactor, operated in the steady-state mode. Bilateral exposure was achieved by a 180-degree rotation at midtime of the exposure. The physical parameters of the free-in-air exposure were an average neutron energy of 0.8 MeV and an average gamma energy of 0.9 MeV, a neutron-to-gamma ratio of approximately 6 to 1, and a dose rate of 0.6 Gy per min. The neutron-to-gamma ratio was achieved by imposing a 15-cm-thick lead wall in front of the reactor core tank wall in the exposure room.

TABLE 1. DEPTH DOSE MEASUREMENTS IN A BEAGLE PHANTOM AND SEVEN BEAGLE CADAVERS

Comparison of Midline to Free-in-Air Doses	Measured in Phantom	Measured in Cadavers ($\pm$ 1 sigma)
TRIGA Reactor		
Midline neutron dose (% air)	28%	34 (12)%
Midline gamma dose (% air)	210%	206 (21)%
Midline total dose (% air)	49%	52 (11)%
Midline neutron-gamma dose ratio	1.1	1.4 (0.5)
Cobalt-60		
Midline gammma dose (% air)	90%	----

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Measurements of dose depth were made at the center of a cylindrical phantom (Table 1). The 15.2-cm diameter of the phantom was approximate to the mean 16-cm diameter determined from the measurement of 54 dog cadavers. The phantom was made of 0.32 cm lucite and filled with muscle equivalent liquid. For mixed neutron-gamma irradiation, the total neutron-plus-gamma dose measured at phantom midline was 49% of that measured free in air; this figure was used to calculate MTD for all dog irradiations. Dose measurements were performed with paired 0.5-cc ion chambers, specifically an A-150 plastic tissue-equivalent chamber with a methane-based tissue-equivalent gas and magnesium chamber with argon gas. Actual animal irradiations were monitored with ionization chambers and sulfur activation foils mounted at fixed positions in the exposure room, to provide corrections for variations in reactor output.

ANTIBIOTIC, FLUID, AND PLATELET THERAPY.

The antibiotics ampicillin, 500 mg, (Polycillin-N, Bristol Laboratories, Syracuse, NY) and gentamycin suflate, 30 mg (Garamycin, Scherring Pharmaceutical Corporation, Kenilworth, NJ) were administered daily until the WBC level reached 1,000/mm^3. Fluid support (lactated Ringer's solution), was administered intravenously as dictated by clinical symptoms. Platelets (3-5 x 10^{10}) obtained by plateletpheresis of donor animals were irradiated with 5,000 rads (cobalt-60 source) and transfused to canines on days 12, 15, and 18 post-irradiation.

RESULTS

LETHALITY OVER A 30-DAY PERIOD (LD50/30) FOLLOWING EXPOSURE TO MIXED NEUTRON-GAMMA IRRADIATION.

Shown in Table 2 are the 30-day mortality values for beagles bilaterally exposed in the AFRRI TRIGA reactor to a range of doses of mixed neutron-gamma radiation. These data placed the LD50(30) value at approximately 1.15 Gy, midline tissue dose. We have not yet been able to accumulate mortality data for cobalt-60 exposure. However, literature values [5-8] place the LD50/30 for

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cobalt-60 bilateral exposure at approximately 2.60 Gy. Thus, based on MTD values the RBE for hemopoietic lethality is approximately 2.26.

TABLE 2. THIRTY-DAY MORTALITY IN DOGS BILATERALLY EXPOSED TO RADIATION[A] AT AVERAGE NEUTRON ENERGY OF 0.8 MEV

Dose (Gy)[b]	Dead/Total	Time of Death (Days)[e]
0.75[c]	0/5	No Death
1.00[c]	0/5	No Death
1.25[c]	4/6	20.0
1.25[d]	0/6	No Death
1.50[c]	5/6	10.2
1.50[d]	4/6	11.0
1.75[d]	4/5	19.0
2.00[d]	3/4	6.5
2.25[d]	4/4	8.0
2.50[c]	4/4	4.0
2.50[d]	2/2	6.0

[a]See Materials and Methods

[b]Midline Tissue Dose. Median Lethal Dose taken to be 1.15 Gy. $LD_{50(30)}$ for cobalt-60 exposure taken to be 2.60 Gy (literature values 5-8)

[c]No support

[d]Support in form of fluids, antibiotics and platelets

[e]Mean time to death for all lethalities

The MTD chosen for a sublethal exposure to cobalt-60 gamma radiation in our experimental protocol for the Combined Injury Program is 1.50 Gy. However, an equivalent MTD of mixed neutron-gamma radiation resulted in an approximate LD85/30. This dose had to be reduced to 1.00 Gy before no lethality was observed. Based on an apparent RBE greater than 2.0 using MTD values, the hemopoietic analysis following exposure to mixed neutron-gamma radiation was conducted at an MTD of 0.75 Gy.

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EFFECT OF 1.50 Gy ^{60}Co or 0.8 MeV NEUTRON RADIATION ON CANINE WBC AND PLATELETS
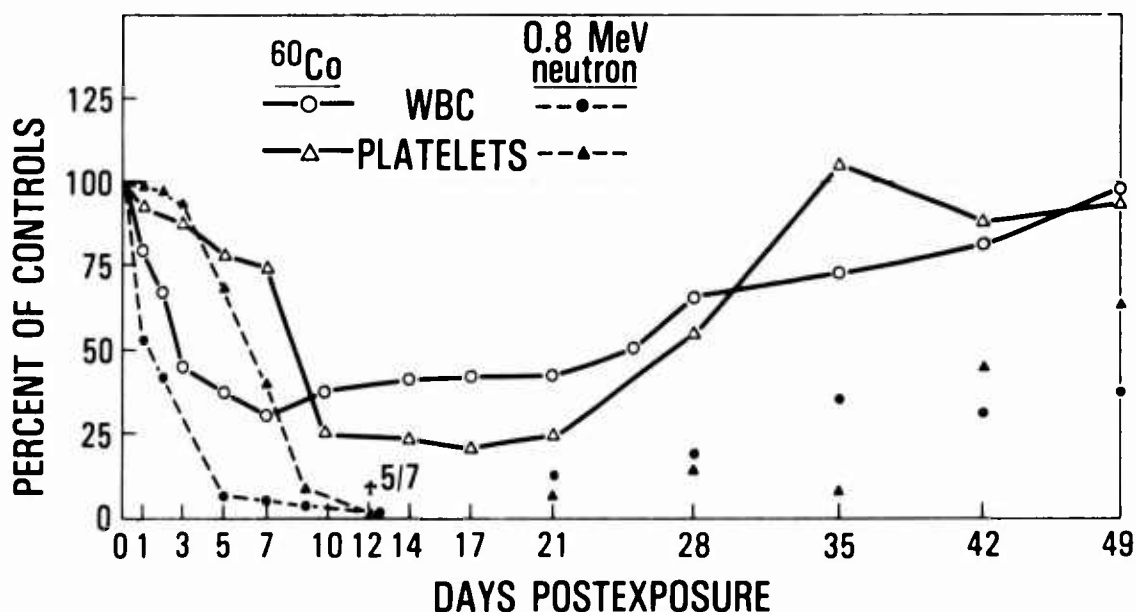

Figure 1. Peripheral blood leukocyte and platelet values as percent of preirradiation values following exposure to either 1.50 Gy gamma or 1.50 Gy mixed neutron-gamma radiation. Mean values 12 dogs (gamma) and 5 dogs (neutron-gamma).

Shown in Figure 1 are the effects of 1.50 Gy cobalt-60 or mixed neutron-gamma radiation on peripheral blood leukocytes and platelets. The 1.50-Gy cobalt-60 gamma radiation significantly reduced circulating levels of white blood cells to a nadir of approximately 30% of preirradiation levels within 7 days postexposure. Platelet levels decreased much slower over the first week and then dropped precipitously to a nadir of 25% of preirradiation levels by day 10. Both parameters (total white cells and platelets) remained depressed through 21 days before recovery toward control levels was observed. Full recovery to preirradiation levels may require 5 to 6 weeks. Also shown in Figure 1 is the respon_^ of these circulating elements to 1.50 Gy of neutron-gamma radiation. It is obvious why this dose level has an LD of 85/30: both components decrease at a greater rate to values less than 10% of normal within 5 and 9 days, respectively, for white cells and platelets, and both continue to decrease until death. The dogs that do survive have shown slow recovery of these parameters, with circulating levels at only 50% of normal by 7 weeks postexposure. It is obvious from the standpoint of equivalent MTD's, that equivalent hematological effects are not observed.

SENSITIVITY OF GRANULOPOIETIC (GM-CFC) AND MACROPHAGE (M-CFC) PROGENITORS TO COBALT-60) AND NEUTRON-GAMMA IRRADIATION.

The survival of GM-CFC and M-CFC to both types of radiation exposure over the dose range of 0.25-3.50 Gy is shown in Figures 2 and 3. Calculation of the dose response over the exponential portion of the survival curves (semi-log plot) yields the D_0 value. This D_0 value is a measure of the relative radiosensitivities of (a) different cell types to radiation of the same quality or (b) the same cell type to radiation of different qualities. This allows a measure of the relative biologic effect of exposure to radiation of different qualities seen between cobalt-60 gamma and TRIGA mixed neutron-gamma radiation. The D_0 is that dose needed to reduce a cell population to the 37% survival level on the exponential portion of the survival curve. D_0 is also a measure of the slope of that particular curve in the exponential region where survival is linearly related to the log function of dose.

DOSE RESPONSE OF BONE MARROW DERIVED GM-CFC
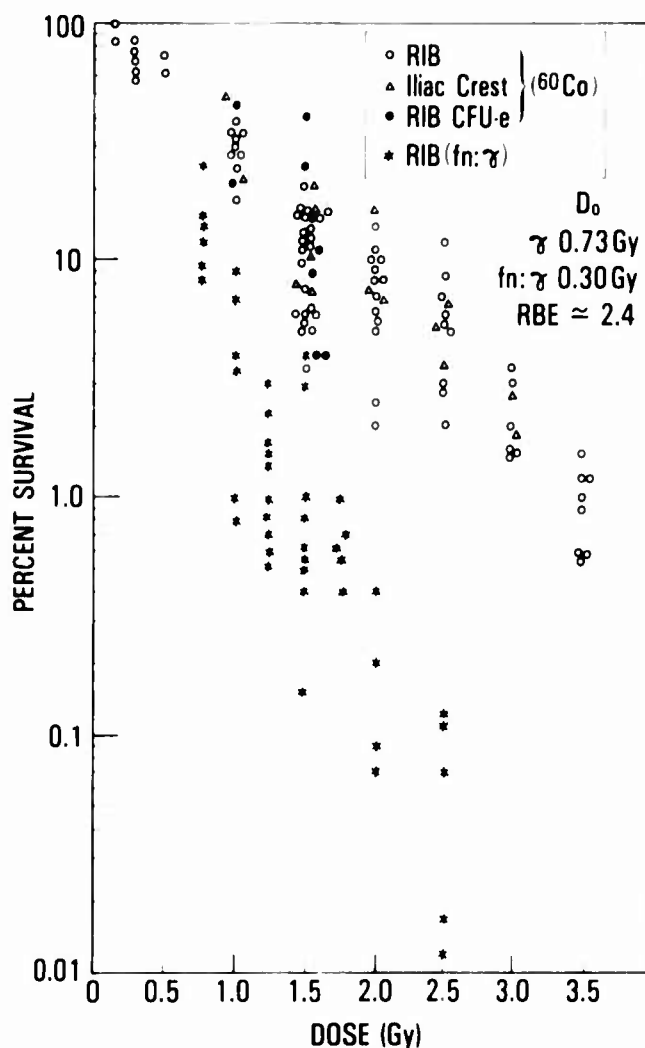**to 60Co or MIXED NEUTRON:GAMMA RADIATION**

Figure 2. Percent survival of GM-CFC assayed 24 hours post-exposure to cobalt-60 gamma or mixed neutron:gamma radiation over the dose (MTD) range of 0.25 Gy to 3.50 Gy.

The respective D_0 values for GM-CFC harvested 24 hours after exposure were approximately 0.73 Gy and 0.30 Gy for cobalt-60 and neutron-gamma radiation, respectively (Figure 2).   The calculation of D_0 values for M-CFC yields a similar response: 0.89 Gy and 0.40 Gy for cobalt-60 and mixed neutron-gamma radiation, respectively (Figure 3).   These hemopoietic responses were again calculated from midline tissue absorbed doses.   Based on these D_0

values, an apparent RBE of greater than 2.0 exists for sensitivity of GM-CFC and M-CFC to mixed neutron-gamma exposure relative to cobalt-60 gamma exposure. Note that the same total MTD of 1.50 Gy of cobalt-60 or neutron-gamma reduced the GM-CFC survival levels to approximately 12% and 0.9%, respectively.

DOSE RESPONSE OF BONE MARROW
DERIVED M-CFC AFTER EXPOSURE to Co-60 or
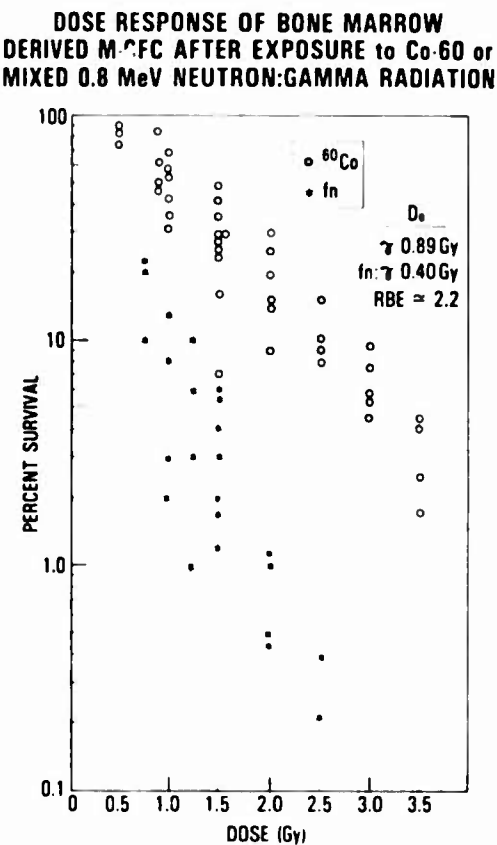MIXED 0.8 MeV NEUTRON:GAMMA RADIATION

Figure 3. Percent survival of M-CFC assayed 24 hours post-exposure to cobalt-60 gamma or mixed neutron:gamma radiation over the dose (MTD) range of 0.25 Gy to 3.50 Gy.

RECOVERY OF BONE MARROW-DERIVED GM-CFC AFTER WHOLE-BODY EXPOSURE TO 1.50 GY COBALT-60 RADIATION.

Exposure of the canine to 1.50 Gy cobalt-60 gamma radiation reduced the GM-CFC levels to approximately 12% of preirradiation values within 24 hours of exposure (Figure 2). This dose level was sublethal, and recovery of the granulopoietic progenitor cells to pre-irradiation levels required approximately 28 to 35 days (Figure 4). GM-CFC levels remained depressed through 5 days, followed by a marked recovery phase from day 7 through day 21. Then, the recovery rate slowed and gradually reached normal levels over the next 2 weeks, within 35 days postexposure.

RECOVERY OF BONE MARROW DERIVED-GM-CFC AFTER 1.50 Gy TBI WITH ^{60}Co or 0.8 MeV NEUTRON
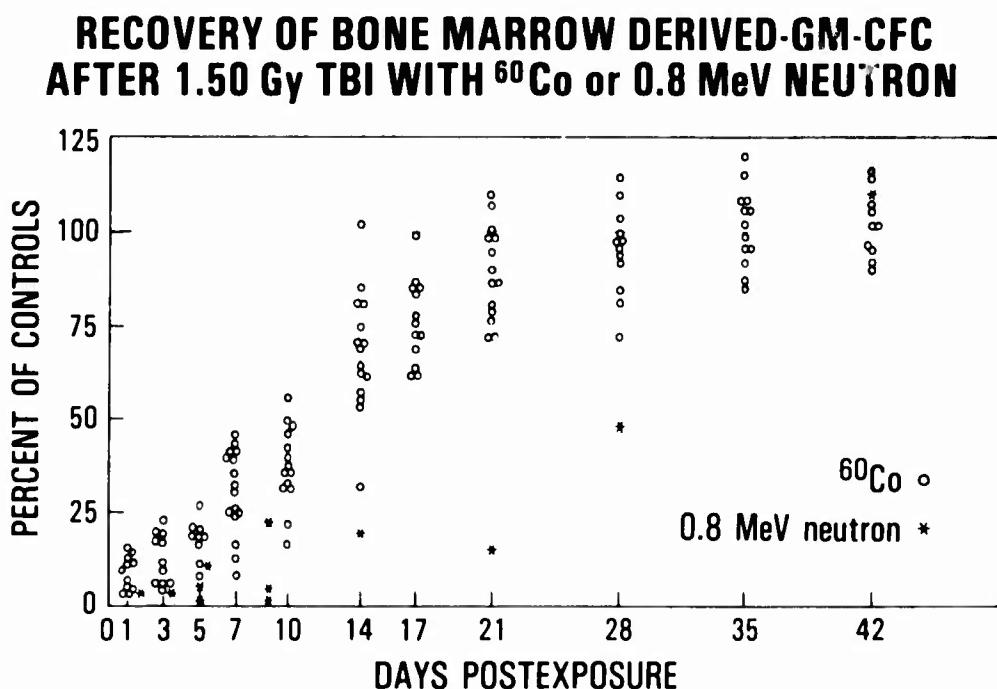

Figure 4. Recovery of bone marrow-derived GM-CFC as percent of pre-irradiation values following exposure to 1.50 Gy gamma radiation. Each value represents individual dog response.

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A DIGRESSION: EFFECT OF GLUCAN ON SURVIVAL OF MICE EXPOSED TO A 100%
LETHAL DOSE OF COBALT-60 RADIATION.

Glucan is a poly-glycan immunomodulator that is isolated from the inner
cell wall of the yeast Saccharomyces cerevisiae, and consists of B 1-3 linked
glucose moieties. When glucan was administered to mice 1 day before whole-
body lethal irradiation (900 rads, LD100/30 cobalt-60), approximately 50% of
the mice survived (>12 months) this otherwise lethal radiation insult (i.e,
100% of controls died by 14 days postirradiation). Experiments are now in
progress to examine the radioprotective effects of glucan when administered
after irradiation.

It appears that the ability of glucan to enhance survival following
irradiation is related to glucan's effects on enhancing hemopoietic recovery.
In general, if gluc'n was administered either 1 day or 1 hour before or 1 hour
after sublethal radiation (650 rads cobalt-60), all hemopoietic progenitors
(CFU-s, GM-CFC, BFU-e, CFU-e, and HSC) recovered 4-7 days sooner in glucan-
treated than in irradiated control mice.[9-11]

DISCUSSION

The groundwork for establishing a combined-injury program is provided by
establishing the variables to be controlled and the animal models that must be
used to answer specific questions about the expression of combined injuries
and the valid extrapolation to human response. In the context of our program
at AFRRI, radiation exposure in the sublethal range is a basic component with
which a secondary insult of physical trauma is combined. It is imperative,
then, that we describe the experimentally essential features of the radio-
biology of our canine model. The dose of 1.50 Gy (MTD, cobalt-60 gamma whole-
body radiation) in the canine was chosen for several reasons: (a) It is sub-
lethal and yet (b) it decreases the granulocyte-macrophage progenitor cells to
a concentra'on less than 12% of normal, and thus provides a significant
hemopoietic stress, (c) STANAG 2083 (NATO Commander's Guide on Radiation
Exposure) defines the Radiation Exposure States (RES) where an exposure
greater than 1.50 Gy is the highest value a soldier can normally receive
without exceeding the emergency risk,[12] and (d) the canine has an approximate

LD50/30 of 2.60 Gy of cobalt-60 radiation (MTD).[5-8] Man has been predicted to have an LD50/30 somewhere between 3.00 and 4.00 Gy, whereas the monkey exhibits an LD50/30 for an average absorbed dose of approximately 5.25 Gy.[13-15] These values place the canine lethality response closer to man; therefore, the canine is an appropriate radiobiological model.

This presentation reports some of the parameters (lethality, hematological value, D_0 value, and hemopoietic recovery) that describe the canine response to radiations of different quality, that is, cobalt-60 gamma and mixed fission neutron-gamma radiation.

LETHALITY AND RBE.

A significant amount of literature establishes the LD50/30 for hemopoietic death of the canine at approximately 2.60 Gy for cobalt-60 gamma irradiation, including X irradiation of the energies 1000 kVp and 2000 kVp [16-20], and an average of 2.28 Gy for exposure to 250 kVp X irradiation.[21-26] These published data indicated a negligible RBE between the cobalt-60 and high-energy X rays but did estimate a small but significant RBE of 0.87 for these higher energy radiations compared to the standard 200-250 kVp X irradation. This value is similar to that reported by Sinclair[27] for LD50/30 values in the mouse, relative to gamma and X irradiation.

Exposure of the beagle to our TRIGA mixed-fission neutron-gamma source resulted in an LD50/30 of 1.15 Gy MTD. This value is significantly lower than the LD50/30 values for fission neutrons of the comparable 1-MeV energy published by Alpen et al.[28] and Ainsworth et al.,[18] with MTDs of 239 rads and 209 rads, respectively. Calculation of the RBE by these investigators resulted in values of approximately 0.90 for Alpen et al. with reference to 250 kVp X irradiation, and 1.38 for Ainsworth et al. with reference to 1 mVp X irradiation. Our observed RBE relative to the standard 250-kVp X-ray exposure would be approximately 2.0, based on the reported average MTD LD50/30 of 228 rads versus our value of 115 rads (an RBE significantly higher than previously reported, as mentioned above). Broerse et al.,[13] however, have

reported a similar RBE for fission neutrons relative to 1-Mvp X irradiation in the primate system. They recorded a total absorbed dose of 2.60 Gy for an LD50/30 from fission neutrons of 1 MeV energy relative to 5.25 Gy for 300-kVp X rays. Thus, the RBE is approximately 2.0 for occurrence of the bone marrow lethality in the rhesus monkey. Similar results have been observed for LD50/30 values in the murine system. Davids,[29] Stewart et al.,[30] and Ainsworth[31] have all reported RBE values of approximately 2.0 for LD50/30 values for exposure to fission spectrum neutrons versus cobalt-60 or X irradiation.

HEMOPOIETIC RESPONSE.

The 1.50-Gy whole-body exposure significantly reduced the circulating blood elements: the white cells, platelets, granulopoietic, macrophage, and erythroid progenitor cells. The nadirs for peripheral blood leukocytes and platelets were observed at 7 days and 10 days, respectively. These levels remained depressed at approximately 25%-35% of preirradiation values through 3 weeks postexposure. Recovery to within normal levels required 5-6 weeks, reflecting the significant although sublethal damage to the marrow stem and progenitor cell pools.

It is this significant and prolonged depression of circulating white cells and platelets that must be considered when selecting medical treatment for the combined-injury patient. The full complement of mature neutrophils, lymphocytes, and platelets will not be available to complete the wound-healing process or resist infections for a critical period of time, depending on dose of radiation and nature of the combined trauma. The medical considerations must be directed not only toward the initial existing condition of the patient but also toward the type of trauma and the situation that will exist 1-3 weeks postexposure.[32]

The reason for the depletion of peripheral blood cells is the sensitivity of their respective progenitor cells and of the pluripotent stem cell populations to the ionizing radiation.[33-36] The sensitivity of the canine GM-CFC and M-CFC, calculated from survival curves over a broad dose range, yield

approximate D_0 values of 0.73 Gy and 0.89 Gy, respectively. The sensitivity of the GM-CFC population measured as number per 10^5 mononuclear cells is consistent with the literature.[33-36] Since we cannot measure the total cellularity of the canine because of experimental design, these values express only the effect of radiation on the relative values of GM-CFC, M-CFC, and CFU-e (per 10^5 MNC) rather than on the absolute number in that marrow location (total per gram of tissue based on total nucleated cells/g of tissue). Wilson et al.,[33] using such a technique for determining the absolute recovery of GM-CFC in weanling beagles, determined the D_0 value to be approximately 70 R following cobalt-60 gamma irradiation. Wilson's results predict that our 1.50-Gy dose reduces the rib marrow cellularity to approximately 60% of normal. Since our D_0 values are similar (73 rads versus 70 R), we could extrapolate our GM-CFC survival fraction to decrease from 12% to roughly 7%, based on total content per gram of rib marrow tissue following 1.50 Gy of gamma radiation.

The RBE based on MTD as observed in our lethality experiments should also be reflected in the radiation sensitivity of marrow progenitor cells, since it is the destruction of these cells that causes the mortality observed over the subsequent 30-day period, known as the hemopoietic syndrome. The calculated RBE's for GM-CFC and M-CFC, as defined by their D_0 values, were indeed greater than 2.0 as observed for the LD50/30. The equivalent biological effect may be viewed from another angle: The respective LD50/30's for gamma (2.60 Gy) and neutron-gamma (1.15 Gy) irradiation should result in a similar percentage sur-vival of marrow progenitor cells. Indeed, the observed value taken from the respective survival curves is approximately 2% survival.

The final aspect of this report is the recovery time necessary for the marrow granulopoietic progenitors to return to preirradiation values. As mentioned above, it is parameters such as this, in addition to the values of the circulating mature cells, that determine the ultimate survival of the patient who encounters a secondary challenge delayed in time from the initial combined-injury or single-radiation insult. Granulopoietic progenitor cells in the canine marrow required 5-6 weeks to reach preirradiation levels. This agrees well with the results of Gerlhartz et al.[34] and Nothdurft and

Fliedner.[35]   These collaborators have performed an excellent series of experiments defining the canine hemopoietic response to a range of sublethal doses of low-energy (300 kVp) X irradiation.   Our data describing the canine response to 1.50 Gy cobalt-60 and mixed-fission neutron-gamma radiations suggest a significant RBE for the fission neutron irradiation. However, these results are based on midline tissue-absorbed dose. The MTD was established by Bond et al.[37] to be used as a relevant reference dose since free in air, entrance, or exit dose have not been acceptable substitu.es. It was their intent to use a value to represent "the dose" received by an animal. This value, MTD, was suggested with full recognition that in irradiating an animal of any size, particularly such as a dog, some degree of inhomogeneity of dose throughout the tissues will exist, no matter what type of radiation is used. In quoting a single value for "dose" received by the animal, it is necessary to fix on the dose received at some fixed location within the animal. Bond states that the ideal is to measure the dose received by a specific critical organ that would correlate with the biological end point, such as the dose received by the bone marrow in the LD50/30 range.   This cannot be done, especially with reference to neutron and mixed neutron-gamma radiation.   In our particular circumstance, we recognize that a complex dosimetric condition exists (Table 2). Our TRIGA exposure of the canine starts with a 6:1 neutron-gamma ratio free in air with an average neutron energy between 0.8 and 1.0 MeV, and it dissipates to a 1:1 ratio at a midline of unknown neutron energy. We do not know (a) the depth-dose response, (b) the absorbed dose to the critical organ, the bone marrow, (c) the spectral changes (neutron energy) as the dose is absorbed, and (d) the resultant change in neutron-gamma ratio with tissue interaction.

However, two technical reports[38,39] describe the depth dose within canine and pig cadavers and the phantoms from mixed-fission neutron-gamma radiation delivered by a TRIGA Mark-F reactor. Considering the reactor identity, the approximate body sizes of the beagle and miniature pig used (approximately 16 cm wide, 8 cm midline), the similar neutron-gamma fields, and the published depth-dose curves taken as percent of total neutron-gamma surface dose, we

take the liberty of calculating the total dose delivered to the canine at approximately 3-cm depth. The 3-cm depth is based on the proposed location of a large percentage of active bone marrow:

Radiation	Biological Effect	MTD(Gy)	TAR	FIA Dose Surface (Gy)	3cm Depth Dose %FIA	3cm Depth Dose (Gy)
n / γ	1%S GM-CFC	1.50	0.49	3.06	75%	2.30
γ	1%S GM-CFC	3.56	0.90	3.96	95%	3.76
RBE		2.37				1.64

These calculations reduce the RBE from 2.37 based on MTD to 1.64 based on extrapolation using depth-dose data from Wingate[38] and Verreli.[39] The 1.64 value is still considerably higher than the previously reported value of 1.38 by Ainsworth et al.,[18] using the same reactor that Wingate evaluted for depth-dose patterns.

SUMMARY

Combined injuries are caused by two or more forms of trauma. In the context of this paper, the primary form of trauma is the exposure to a sublethal dose of ionizing radiation. The combination of a subsequent sublethal exposure to mechanical or thermal trauma can change two individually sublethal events into a lethal response for the combined-injury host. The establishment of a canine model for radiation-induced aplasia and bacterial sepsis will allow us to investigate the mechanisms involved in mediating the cellular and humoral defenses against sepsis in the irradiated and traumatized host. The large-animal model is also appropriate for assessing the immunologic, pharmacologic and surgical modes of intervention following combined injuries. This paper describes the radiation-induced suppression and recovery of the canine hematopoietic system.

Pure-bred, male and female, young adult beagle canines (weighing 9-12 kg) were used throughout this study. They were bilaterally exposed to either cobalt-60 irradiation (at a dose-rate of 0.1 Gy per minute) or to mixed

neutron-gamma radiation in the AFRRI TRIGA (reactor at a dose-rate of 0.6 Gy per minute) to a predetermined total midline tissue absorbed dose (MTD). The neutron-gamma ratio free in air at the skin surface is approximately 6:1, which dissipates to approximately 1:1 at midline tissue, with an average neutron energy of 0.8 MeV.

The parameters measured were lethality, peripheral hematologic changes, D_0 values for GM-CFC and M-CFC, and recovery of the hemopoietic system following exposure to 1.5 Gy cobalt-60 gamma radiation.

Exposure to 1.5 Gy cobalt-60 radiation was sublethal for 100% of the dogs, and doses of 1.5 Gy and 1.25 Gy in the mixed neutron-gamma field resulted in approximately 85% and 67% lethality, respectively. Exposure to 0.75 Gy was 100% sublethal.

Exposure of dogs over a dose range of 0.50-3.50 Gy of either gamma or mixed neutron-gamma radiation resulted in significantly different D_0 values within the GM-CFC and M-CFC populations. The results show D_0 values for gamma exposure of 0.73 Gy and 0.89 Gy for GM-CFC and M-CFC, respectively. Exposure to mixed neutron-gamma radiation reduced these values to 0.30 Gy and 0.40 Gy, respectively. Calculated RBE values for equivalent biological end-points relative to MTD's for GM-CFC was 2.4 and for M-CFC was 2.2. An approximate biologically equivalent dose for the 1.50-Gy cobalt-60 radiation was taken as 0.75 Gy for neutron-gamma exposure. Hemopoietic recovery in dogs exposed to 1.50 Gy gamma radiation took 4-5 weeks to return to preirradiation levels.

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DISCUSSION PERIOD WITH DR. MacVITTIE

DR. BAUM: I think you ought to clarify your RBE calculation in dogs. Neutrons attenuate rapidly compared to gamma radiation in larger dogs before they get to the midline and thus represent a near uniform exposure. Years ago Drs. Bond and Alpen estimated an RBE of one for the dog neutron to gamma using exposures similar to yours. My question is , do you know the actual dose to the bone marrow before you estimate an RBE of 2.3? You get a lot more neutron radiation to the bone marrow as compared to the midline because of the bone marrow's anatomical location. So probably the RBE is somewhere between one and 2.5. How did you calculate it? Did you use 0.66 of the dose free in air for your midline tissue dose?

DR. MacVITTIE: Yes. However, the tissue to air ra io for our exposures was approximately 0.49 if I am not mistaken.

DR. BAUM: Until you know the actual bone marrow dose it is difficult to determine the true RBE. The midline tissue dose is useful for RBE calculations if restricted to situations in which relatively uniform dose distribution exists. For hematological death, the dose to the bone marrow, if obtainable, would be more appropriate.

DR. MacVITTIE: Your question is very valid and this is one of the areas that is of great concern at AFRRI. We hope to be able to determine the depth dose as as well as neutron energy at various anatomic sites in the near future. That 0.8 MeV value is the average energy of the neutron in air at the surface; the dissipation of it through the tissue is not known yet.

If we use the calculations of depth dose from the TRIGA provided by Verrelli in the pig and Wingate in the dog for a dose at 4 cm depth, we can reduce the RBE to approximately 1.64. Still significantly higher than the value of 1.0 by Bond and Alpen and 1.38 by Ainsworth.

DR. CONKLIN: We have a contract for the development of Monte Carlo computational capability to give us not only dose, but spectral information at any organ site of interest, not just the bone marrow. Hopefully that will be in place within the next 12 months.

UNKNOWN: When you spoke of the dog irradiation, the neutron to gamma ratio was about 6 to 1. Do you expose your dogs unilaterally or bilaterally?

DR. MacVITTIE: Exposures are unilateral, but recipients are rotated at mid-dose so that the other side does get exposed.

DR. McCOY: Have you tried post irradiation treatment with glucan? Studies with interferon show a rapid return of immune competence as related to natural killer cells following lethal cobalt irradiation. Our data suggested there was a significant increase in survival of the animals irradiated and then treated with interferon 24 hours later.

DR. MacVITTIE: To date, most of the data has to do with injecting the glucan prior to exposure, but we have some data post-
exposure. Dr. Patchen is in the audience, perhaps she would like to respond to your comment.

DR. PATCHEN: Data that Dr. MacVittie showed were responses to particulate glucan. Particulate glucan administered after irradiation, is not effective, partially because it appears to sensitize animals to endotoxin endogenously released after high doses of radiation. Soluble glucan, which we have just recently obtained, does not sensitize to endotoxin and it looks like it is going to be effective before and after irradiation.

DR. CAMP: Is someone at your shop talking to the operational commanders about the necessity of individual radiation detectors, because right now I think there are only two in the Battalion. It is futile from a medical treatment standpoint to depend on that area dose if you are looking at an individual. The question is, is somebody talking to the big guys up there?

DR. MacVITTIE. Jim, do you want to answer that?

DR. CONKLIN. We go through this every time we make our pitch each year. I think Major Pete Meyers is here from the U.S. Army Nuclear and Chemical Agency and they are well-aware that two dosimeters are issued per platoon. It is a subject of discussion at every NATO meeting that we have. Our allies, I think very correctly, feel everyone should have a dosimeter. That is our feeling. We articulate it every chance we have. It has not been accepted by TRADOC as standard for the U.S. Army, nor for any of the services.

DR. BAINES: In situations where you have either a toxic dose of a chemical or one hit kinetics with irradiation, you may knock out a sensitive population of cells and a resistant population may then repopulate. If an animal has been irradiated one time, and allowed to recover, upon second radiation would you expect to see the same kinetics for recovery as you did in the first case?

DR. MacVITTIE: No, There has been a good deal of information done on "residual damage" to the hemopoietic system. Dr. Baum, has shown that if you exposed rats to 300 rads, of ^{60}Co ionizing radiation and wait a period of time, before exposing them again, residual damage is such that the rat cannot respond nearly as well to the second challenge. The same question is relevant with respect not only to radiation, but as I have tried to indicate, in response to other types of combined injuries.

DR. CONKLIN: I have a question for Dr. Santos. Dr. Santos, in the bone marrow transplant patients at Hopkins who get nominally 1,000 rads whole-body radiation, have you evaluated the T-lymphocyte population as being functional or afunctional post-irradiation?

DR. SANTOS: In autologous or identical twin transplants, we have only done about 30 identical twins, there is little residual host immunity with normal T-cell subsets very early. As they come back what we see is something that is very akin to the recapitulation of ontogeny. Cells possess markers, but are afunctional.

I think the allogeneic system, although similar, is much more complicated because of other things. We have not studied sublethal radiation to see if it, in point of fact, performs similarily.